A NOTE ON A UREDOSPORE COLOUR MUTANT IN BARLEY LEAF RUST, *PUCCINIA HORDEI* OTTH.

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(Two Text-figures.)

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Synopsis.

A colour mutant of *Puccinia hordei* Otth. is described. It differs from the original rust in certain morphological and physiological characteristics but behaves the same in pathogenicity on differential varieties used in race identification. This mutation will enable competition trends with other races on susceptible varieties to be more readily studied.

Introduction.

Pathogenic changes presumably due to mutation in the absence of sexual recombination are common in cereal rusts. Changes in visible morphological characters are less common, but colour mutations have been recorded in the case of wheat stem or leaf rust by Waterhouse (1952), Johnson, Newton and Brown (1934), and Johnston (1930).

Waterhouse reported that on seven occasions cadmium yellow or light cadmium uredopustules of *Puccinia graminis tritici* E. and H. had been noticed among normal coloured pustules. In six of these cases the race determinations showed that the light coloured culture was the same as the parent culture, but in the seventh instance the colour mutation proved to be a different race, namely r.56, which arose from a stock culture of r.34, and it is interesting that Waterhouse reported that when teleutospores of r.34 were used to infect the barberry, it proved to be heterozygous, one of the derived races being 56.

Johnson *et al.* studied the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici.* Although the cytoplasm was important in the inheritance of pathogenic differences there was every indication that spore colour was Mendelian in character. Red spore colour appeared to be due to the interaction of two dominant factors (one for orange pigment in the cytoplasm, and one for greyish-brown pigment in the spore wall). White colour was explained by the presence of the recessive allelomorphs of both factors.

Johnston collected an aberrant race, later described as race 27 of *P. triticina* Erikss., which differed from other known races in pustule size and colour, spore size and shape, a longer incubation period, thickness of spore wall and host reactions.

Description of Mutant.

Colour.

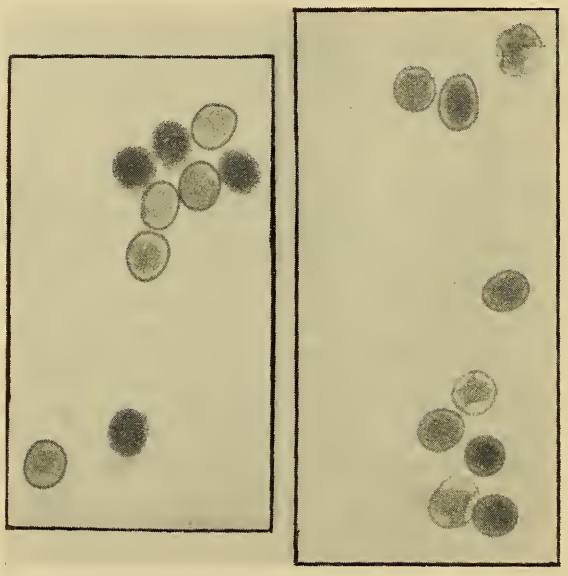
Whilst counting F_2 segregates for the inheritance of resistance to race UN14 of barley leaf rust the senior author observed a pustule which was of a bright yellow colour compared with the typical brown of this race. This pustule was separated and inoculated onto seedlings of a susceptible host. Although not free of contamination from this transfer, pure cultures of the yellow mutant were obtained in the succeeding transfer. Under the microscope and mounted in water, the cytoplasm of the mutant spores was paler yellow than that of the normal rust.

When compared with the colour charts in Ridgway's "Color Standards and Color Nomenclature" the mutant rust pustule approximated most closely "Cadmium Yellow" (Plate III) whilst the original was best described as "Sanford's Brown" (Plate II).

Morphology.

In morphology the two rusts were quite distinct. The uredospores of the original were globose, ellipsoid or oval, with few round, whilst those of the mutant were rounder, being mostly globose or round, with few oval. Differences in spore size were also shown. Measurements of mature spores shaken onto clear lacto-phenol on a slide were made, 100 spores being included in each sample. The spores of the original cultures were 25·87μ ± 1·94μ long, and 23·08μ ± 1·37μ wide, whilst the respective measurements for mutant spores were 24·37μ ± 1·64μ and 22·77μ ± 1·28μ. At the P = 0·01 level, differences in spore length were significant. However, differences in spore width were not significant at either the P = 0·05 or P = 0·01 levels.

Spores of both types were minutely echinulate. The original spores had 2–4 moderately large germ pores on each face, whilst those of the mutant were distinctly smaller and less visible. The spore walls of the former were considerably less hyaline. Certain features of the cytoplasm are later discussed.



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Text-figures 1-2.

1.—Photomicrograph of uredospores of the original and mutant rusts of *Puccinia hordei* Otth., taken 5 minutes after staining with acid fuchsine in lacto-phenol. The mutant spores are intensely stained, whilst those of the original rust are unaffected. The distinctness and darker colour of the spore wall of the latter are exhibited in unstained preparations as well. (× 240. Orthochromatic plate.)

2.—Photomicrograph of uredospores of the original and mutant rusts of *Puccinia hordei* Otth., in clove oil after treatment for two minutes with acid fuchsine in lactic acid. Spores of the mutant type rust (with stained cytoplasm) are unaffected. Shrinkage of the cytoplasm and, to a lesser extent, the walls of the original rust spores has occurred giving an irregular outline. (× 300. Orthochromatic plate.)

Physiology.

When the mutant and original rusts were inoculated onto the same leaf of a susceptible barley variety, pustules of the original race appeared 1–2 days earlier than those of the mutant. Secondary smaller uredosori in a circle around the original pustule appeared to be more readily formed in the case of the mutant rust.

To ascertain whether possible physiological differences between the two rusts were reflected in differential staining reactions, certain tests of this nature were carried out. In general, little difference was observed when basic dyes were used. However, with acid dyes in solution with certain organic acids, markedly differential results were

obtained. With acid fuchsine in lactic acid or lacto-phenol cotton blue the cytoplasm of the mutant spores was darkly stained after 5 minutes, whilst that of the original spores remained unaffected (see Text-fig. 1). After an interval of 15 minutes, however, the latter gradually became darker, and ultimately became as intensely stained as the mutant type.

A further physiological difference was that of apparent osmotic value in the two types of spores. This was measured by the relative plasmolysis in sucrose solutions of varying molarities. Where plasmolysis occurred, mature spores were plasmolysed within 5 minutes. With a sucrose solution of 0·52 mol. (or approx. 18 atm.) the spores of the original rust were plasmolysed, whilst those of the mutant type were unaffected. This differential behaviour ceased to exist at 0·60 mol. (approx. 20 atm.), when both types were plasmolysed.

A differential effect, apparently similar to plasmolysis, and similar in many respects to that shown by meristematic plant cells, was also observed upon treatment with clove oil. Mutant type uredospores were less affected by this treatment and when clove oil was added to spores, previously treated for two minutes with acid fuchsine in lactic acid, this difference became more noticeable. Shrinkage of both the unstained cytoplasm and, to a lesser extent, the walls of the original rust spores occurred rapidly, giving them ultimately an irregular shape. Mutant type spores remained stained but unaffected by this treatment (see Text-fig. 2).

Pathogenicity.

Another aspect investigated was that of possible pathogenic changes associated with the colour variation. Both rusts were tested side by side under comparable conditions in the seedling stage on the differential set devised by Levine and Cherewick (1952). The behaviour on varieties of the differential set was identical with both rusts, and is as under:

Variety.	Reaction Type.	Variety.	Reaction Type.
Reka I C.I.5051	3^{+c}	Club Mariout C.I.261	2^{+c}
Sudan C.I.6489	4	Samaria C.I.6493	4
Cruzat C.I.6482	;, 1^{-}	Berg C.I.6486	4
Chilean D C.I.1433	2	Gold C.I.1145	3^{c}
Bolivia C.I.1257	2^{+}	Lechtaler C.I.6488	3^{+}
Oderbrucker C.I.940	4	Austral C.I.6483	;
Quinn C.I.1024	;	Kinver C.I.2361	4
Egypt 4 C.I.6481	4	Speciale C.I.7536	4

From these reactions both rusts conform most closely to unified-numeration (UN) race number 14 on the key described by Levine and Cherewick. Minor reaction differences on varieties such as Bolivia (1^{++} compared with 2^{+} in the present investigation) are acknowledged, but on the broad basis of resistance and susceptibility the rusts are keyed out to this race.

Discussion.

In the absence of genetic recombination associated with the sexual stage on the alternate hosts, *Ornithogalum* spp., which obviously could play no part in the present instance under glasshouse conditions, some other phenomenon must be sought to explain the origin of the colour variant. Nuclear recombination, associated with heterocaryosis, and mutation are other possible mechanisms to account for variability in rusts. The role of heterocaryosis at the present time awaits further confirmatory evidence, and the most logical explanation for the colour change is mutation used in the broadest sense to include both gene changes or chromosomal aberrations.

Apparent mutation for pathogenicity frequently occurs in many cereal rusts. These are physiological changes with usually no readily discernible morphological effects. This phenomenon is considered to account in the main for the many changes in both the wheat stem and leaf rust organism under Australian conditions. More specifically, in the case of the organism causing barley leaf rust, a major change in pathogenicity was detected in 1952 locally, when, in the field, formerly resistant varieties used in genetical

studies became susceptible to a new variant. On the differential set a change from race UN16 to race UN14 was indicated. In this pathogenic change, also, no evidence is available to suggest that genetic recombination was responsible since the alternate hosts are confined entirely to a few locations in gardens, where they have never been observed carrying the aecidial stage.

In the present instance certain morphological and physiological changes were associated with that of colour. This might suggest that more than a point or single gene mutation was involved. However, pleiotropic effects of a single gene are widely known in higher organisms and, in addition, Johnston (1930) found other characteristics, many similar to those observed in the present study, which were associated with a change in uredospore colour. Although there was no direct proof that Johnston's variant arose through mutation, this was considered a likely probability in view of the complete absence of the sexual stage of *P. triticina* in nature in North America. Johnston observed an associated change in pathogenicity in addition. No such association was evident in the present study. However, varieties outside the differential set were not studied in any detail, and the evidence for absence of change in pathogenicity is not completely substantiated. In this connection it might be mentioned, however, that F_2 segregates, where tested, behaved identically with both the original and mutant rusts.

One obvious value of this mutant will be to enable competition trends between it and normal coloured rusts to be accurately assessed in successive transfers on apparently fully and uniformly susceptible barley hosts. There is nothing to suggest that such a mutation may not have occurred in nature before. It is logical to consider that the longer incubation period of the mutant would be an aspect placing it at a selective disadvantage in nature. The competitive behaviour in such cases can be readily studied under glasshouse conditions to gain knowledge of the possible evolutionary trends in nature. It was immediately observed that the newer UN race 14 was more virulent, and rapidly superseded the previous omnipresent UN race 16 under field conditions on commercial varieties susceptible to both rusts. Unpublished data from competition studies in the glasshouse have confirmed this observation, but in the absence of a readily contrasting morphological character such as uredosorus colour such studies are considerably more difficult technically.

Another obvious advantage given by the mutant rust is that in investigations on the mode of inheritance of resistance, identical plants in segregating populations can be studied simultaneously with more than a single race.

Acknowledgements.

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